Received: 21 May 2023, Accepted: 07 July 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 6, 1227-1233

Cross Sectional, Seroprevalence Study of Peste des Petits Ruminants and the Related Risk Factors During Outbreak in Goats' Farm in Egypt

Halla E.K. El Bahgy[1], Hala K. Abdelmegeed[2], Dina A. Abdelwahed[2], Rabab T. Hassanien[2], Ahmed F. Afify[2*], Omnia M. Kattab[3], Momtaz A. Shahein[2]

[1]Veterinary Hygiene and Management Department, Faculty of Veterinary Medicine, Benha University, Moshtohor, 13736, Egypt.

[2]Virology Research Department, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), 12618, Giza, Egypt.

[3]Genome Research Unit, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), 12618, Giza, Egypt.

***Correspondence**
Corresponding author: Ahmed F. Afify
E-mail address: Fieldagy_ahmed@yahoo.com

Abstract

Peste des Petits ruminants' virus (PPRV) is a notifiable transboundary and economically significant viral disease that affects goats and sheep. The current study was conducted to identify the seroprevalences of PPRV in goat farm in Marsa-Matroh province, Egypt during an outbreak in 2022. Moreover, this work aims to study the relevant risk factors directly related to the virus infection and attempt molecular characterization and phylogenetic analysis of the circulating strain. The samples included 356 sera, 154 nasal swabs, and 10 tissue samples were collected for PPRV screening and molecular characterization. The seroprevalence percent was found of 42.69%. It was observed that the prevalence rate, and case-fatality rate were higher under 6 months of age than in adults, and referring to sex, females had a more significant disease incidence than males. Based on phylogenetic analysis; the strains of the current study: PPR/AHRI-Matrouh1/Egy/2022 (accession number: OP881991) and PPR/AHRI-Matrouh2/Egy/2022 (accession number: OP881992) were identified as PPRV lineage IV, with 99.2% and 98.8% identity to the Ethiopian strain (Accession number MK571524) and Sudanese strain (Accession no HQ131931) respectively. An efficient PPR vaccination program with rigorous quarantine measures at the borders is advised to be implemented in the country to control the spread of the disease and avoid the entry of novel strains into the Egyptian governorates.

KEYWORDS
PPRV, Retrospective study, Risk factor, Seroprevalence, Phylogenetic analysis.

INTRODUCTION

Peste des Petits ruminants' virus (PPRV) is a highly contagious viral disease that infects both domestic and wild small ruminants and has an economic impact on agricultural sustainability in endemic regions (Mahapatra *et al*., 2021). Currently, PPR is classified as a significant transboundary and notifiable disease that poses an emerging or re-emerging concern in several countries around the world due to the risk of fast spread and the resulting limitations on international trade in animals and animal products (Ahaduzzaman, 2020; Mulumba-Mfumu *et al*., 2021).

PPR is a rinderpest-like illness that affects goats and sheep. It is also known as ovine rinderpest, goat plague, a plague of small ruminants, or Kata (Dubie *et al*., 2022). PPR severity is characterized as per acute, acute, subacute, or subclinical based on the disease's viral virulence and predisposing variables. The most frequent type of PPR is the acute type; it causes high fever, ulcers in the mouth with secretions, nasal discharges, pneumonia, severe diarrhea, and death in some cases. In epidemic regions, the morbidity rate of PPR is estimated to be 10%- 90%, with a fatality rate of 50%-90% (Wagdy *et al*., 2018; Alemu *et al*., 2019; Ahmed *et al*., 2021). Goats were reported to show more severe symptoms and outbreaks than sheep (Nafea *et al*., 2019). It has also been reported that subclinical forms of the disease may develop in other large ruminants (cattle and buffalo) and pigs, which play an essential role in disease propagation and circulation (Nafea *et al*., 2019).

The virus is classified as family paramyxoviridae, subfamily Orthoparamyxovirinae, genus Morbillivirus (ICTV, 2018). The virus has enveloped with non-segmented, negative sense and single-stranded RNA of about 15,948 nucleotides in length (Chard *et al*., 2008). PPR viral genome has six genes that encode for six structural PPRV proteins (the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the haemagglutinin protein (H), and the large polymerase protein (L)) and two non-structural proteins, V and C (Munir *et al*., 2012).

The disease transmission occurs through aerosol by close contact between infected and vulnerable animals, and it sheds after a short time of infection. Aerosols transmitted via nuzzling and licking between infected and susceptible animals, as well as oral routes or contact with infected animals' fluids and excretions, are considered primary transmission modes (Fournié *et al*., 2018; Halecker *et al*., 2020).

PPRV has been identified as a significant concern, with over one billion small ruminants in Africa and Asia at risk of infection. It was recorded for the first time in western Africa on the Ivory Coast (Ahmed *et al*., 2021). The virus has recently become widespread in Africa and specific areas of Asia and poses a threat to other continents like Europe (Parida *et al*., 2016). Based on the phylogenetic analysis of nucleoprotein (NP) and fusion genes (F)

ISSN: 2090-6277/2090-6269/

sequence analysis for particular geographical regions, PPRV has been classified into four genetically distinct lineages (I-IV) (Dilli *et al*., 2011). The four lineages have historically been dispersed geographically as follows: lineages I and II were found in western and central Africa; lineage III was located in eastern Africa and the southern Middle east; and lineage IV was spread throughout the Middle East and southern Asia (Ahmed *et al*., 2021).

Concerning the recent global movement towards preventing, controlling, and eradicating PPR, there is a crucial need to raise global awareness about the disease benefiting from the regulations outlined in the national feedback platforms (FAO, 2015). Considering this, it is evident that there are considerable gaps in knowledge concerning the epidemiology of PPR in Egypt. These gaps include the following questions: 1. what is the extent of the disease perpetuation among the susceptible domesticated hosts? 2. What are the lineages of the viruses circulating among the susceptible animals, goats, as a model? 3. And finally, what are the possible factors influencing the disease to perpetuate among these animals?

Since PPR is on the World Organization for Animal Health (OIE) list of notifiable diseases and is currently a global threat to the animal industry, it needs more attention. The setbacks mentioned earlier observed in Egypt, coupled with the animal species existing in the country, the rearing pattern practiced in the country with the absence of regular vaccination records, have promoted the authors to conduct this cross sectional besides retrospective investigation about the prevalence of the disease, which is probably, the first in Egypt. Also, to analyze the risk factors related to PPRV control development and implementation, then perform molecular characterization and phylogenetic analysis of the current circulating strain of PPRV.

Answers given to the previously raised questions would expect to offer baseline data on the disease and help establish effective, sustainable preventive and control programs.

MATERIALS AND METHODS

Ethics statement

The Local Ethics Committee of animal experiments at the Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Egypt, has approved the sample collection in this study following institutional, national, and international guidelines with protocol number ARC-AHRI-23-04.

Samples collection and preparation

A suspected outbreak of PPRV was investigated in a goat farm in Marsa-Matroh province, Egypt, in 2022. The flock was non-vaccinated and animals were suffered from clinical signs suspected to be PPRV infection as diarrhea, dullness, nasal discharge, fever, and salivation.

Sera samples

A total number of 356 blood samples were collected through this study. The collected samples included 145 males, and 211 females, with ages ranged from less than six months (n= 102) and between 2-3 years old (n=254). The samples were collected from the jugular vein in clean, dry centrifugal tubes, left to clot, and then centrifuged at 4°C at 3000 rpm for 10 minutes. The clear serum was obtained by using sterile Pasteur pipettes and placed in Eppendorf tubes, labeled, and stored at -20°C to be used for PPRV antibodies detection using the ELISA test.

Nasal swab samples

A total number of 154 nasal swab samples were collected from animals with clinical signs suspected to PPRV infection. Forty-four samples were collected from young animals between 1-6 months of age, and 110 samples were collected from adult animals whose ages ranged between 2-3 years old. The samples included 63 males and 91 females. The nasal swabs were collected by inserting a sterile swab deeply into the cavity. The cotton area of the swab was separated gently from the swab stick using sterile forceps and scissors. The swab was put into a sterile Eppendorf tube with 1.5 mL of sterile phosphate buffer saline (PBS; 0.01 M pH 7.4). The swab was ultimately squeezed in the PBS and centrifuged at 10,000 RPM for 3–5 minutes at 4°C. The supernatant was collected and stored at -70°C till further analysis.

Tissue samples

Intestine, and lung tissue were collected post-mortem from 10 animals that were suffered from clinical signs suspected to PPRV infection.

The samples were prepared by making 10% homogenate of infected tissues in sterile phosphate-buffered saline (PBS; 0.01 M pH 7.4) (w/v). Then, the suspension was centrifuged at 10,000 RPM for 5 minutes at 4°C in a 1.5 mL Eppendorf tube, and the supernatant was collected and stored at −70°C till further analysis.

Detection of PPRV antibody

The prepared sera samples were tested for the presence of anti-PPRV nucleoprotein (NP) antibodies using ID Screen® PPRV competitive ELISA kit (IDvet, rue Louis Pasteur, Grabels, France) according to the manufacturer's instruction (Libeau *et al*., 1995).

Detection of PPRV antigen

Nasal swab samples were tested for the detection of PPRV antigen by using ID Screan® antigen capture ELISA kit according to the manufacturer's instruction (Abubakar *et al*., 2011). This ELISA is a sandwich ELISA assay that uses an Anti-PPRV NP as capture antibodies and an anti-PPRV-NP monoclonal-HRP antibody as detecting antibodies.

Molecular detection of PPRV

RNA extraction

Total RNA was extracted from all collected tissue samples (10) using the QIAamp viral RNA Mini kit (Qiagen, Germany, GmbH). Briefly, 140 µl of the sample suspension was incubated with 560 µl of AVL lysis buffer and 5.6 µl of carrier RNA at room temperature for 10 min. After incubation, 560 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with the kit's 60 µl of elution buffer.

PCR amplification

The PCR protocol used in the current study to amplify 351 bp of the Nucleo-protein (NP) gene of PPRV in all tissue samples, as previously described by Kgotlele *et al*. (2014). A 25 µl reaction volume was prepared as fellow: 12.5 µl of Quanti-tect probe RT-PCR buffer (Qiagen, Gmbh), 1 µl of forward primer

(5′-TCTCGAAATCGCCTCACAGACTG -3′), and 1 µl of reverse primer (5′-CCTCCTCCTGGTCCTCCAGAATCT -3′) (20 pmol), 0.25 µl of RT-enzyme, 5.25 µl of water, and 5 µl of extracted RNA. PCR tubes were placed in a Biometra™ thermal cycler. Reverse transcription was applied at 50°C for 30 min, and a primary denaturation was done at 95°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 40 seconds, and 72°C for 40 seconds. A final extension was done at 72°C for 10 minutes.

Analysis of the PCR Products

The PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products were loaded in each gel slot. A general 100 bp ladder (Fermentas, Germany) was used to determine the fragment sizes.

Sequencing and phylogenetic analysis

The purified PCR products of two representative positive samples were sequenced directly using the ABI PRISM Big Dye Terminators v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The products of the sequencing reactions were cleaned-up using a Centrisep purification kit (Applied Bio-system, CA-USA). The purified products were sequenced directly using an ABI PRISM3500 genetic analyzer (Applied Biosystems).

BLAST® analysis (Basic Local Alignment Search Tool) (Altschul *et al*., 1990) was initially performed to establish sequence identity to GenBank accessions. The phylogenetic tree was created by the MegAlign module of Lasergene DNAstar version 12.1 (Thompson *et al*., 1994), and Phylogenetic analyses were done using maximum likelihood, neighbor-joining, and maximum parsimony in MEGA6 (Tamura *et al*., 2013).

RESULTS

Detection of PPRV antibody detection

Out of three hundred and fifty-six total collected samples, one hundred and fifty-two samples were positive for the presence of the PPRV antibodies (152/356) with a percentage of 42.96%. Regarding the age, the positive detected animals (152/356) were 48 animal (48/102) less than 6 month old, whereas 104 animal (104/254) were between 2-3 years old, with percentage of 47.05%, and 40.94% respectively. Also, regarding the sex; the positive animals (152/356) were 51/145 males, and 101/211 females with percentage of 35.17%, and 47.86% respectively (Table 1).

Detection of PPRV antigen detection

Out of 154 nasal swab samples, 142 samples were positive (92.20%). The positive samples percentage was greater in young animals with less than 6m of age (43/44, 97.72%) than adult animals aged between 2-3 years (99/110, 90%). Furthermore, females (87/91, 95.60%) had a higher incidence rate than males (55/63, 87.30%).

In terms of the case-fatality rate, the proportion was 93.66%. The case-fatality rate was found in animals younger than 6m old animals 100%, and 90.90% for adults aged 2-3 years. Male and female goats had death rates of 92.72% and 94.25%, respectively (Table 2).

Molecular detection of PPRV

The NP gene was successfully amplified in all 10 tested samples; all samples were found positive to have a specific molecular weight band at 351 bp.

Sequencing and phylogenetic analysis

Partial nucleotide sequence of the PPRV-NP gene for 2 representative tested samples named PPR/AHRI-Matrouh1/Egy/2022, and PPR/AHRI-Matrouh2/Egy/2022; has been submitted to the Genbank with accession numbers OP881991 and OP881992 respectively. Based on the phylogenetic analysis, the two samples were identified as PPRVs. According to the sequencing data analysis, the strains of the current study were 99.2% closely related to the Ethiopian strain (Accession number MK571524, 2011). Furthermore, our strains have 98.8% and 98.4% similarity to the reference strains from Sudan (Accession no HQ131931, 2016 and MK371449, 2015) respectively.

Comparing our strains with the Egyptian strains from the GeneBank, our strains exhibited 99.2% identity to other strains from Ismailia (Accession number: JN202923, 2010, JN202924, 2010 and JN202926, 2014) and 98.8% identity with strains from

Table 1. The Seroprevalence of PPRV antibodies in non-vaccinated goat flocks in Marsa-Matroh governorate, Egypt.

Risk factors		No. of samples	Seroprevalence rate	
			No of positive	%
Age	Young (<6 months)	102	48	47.05
	Adult (2-3 years)	254	104	40.94
Sex	Male	145	51	35.17
	Female	211	101	47.86
Total		356	152	42.69

Table 2. The incidence rate and case-fatality rate of PPRV antigen infection in the goat samples collected from Marsa- Matroh governorate according to age and Sex.

Risk factors		No. of samples	Incidence rate		Case-fatality rate	
			No of positive	%	No of positive	%
Age	Young (<6 months)	44	43	97.72	43	100
	Adult (2-3 years)	110	99	90	90	90.9
Sex	Male	63	55	87.3	51	92.72
	Female	91	87	95.6	82	94.25
Total		154	142	92.2	133	93.66

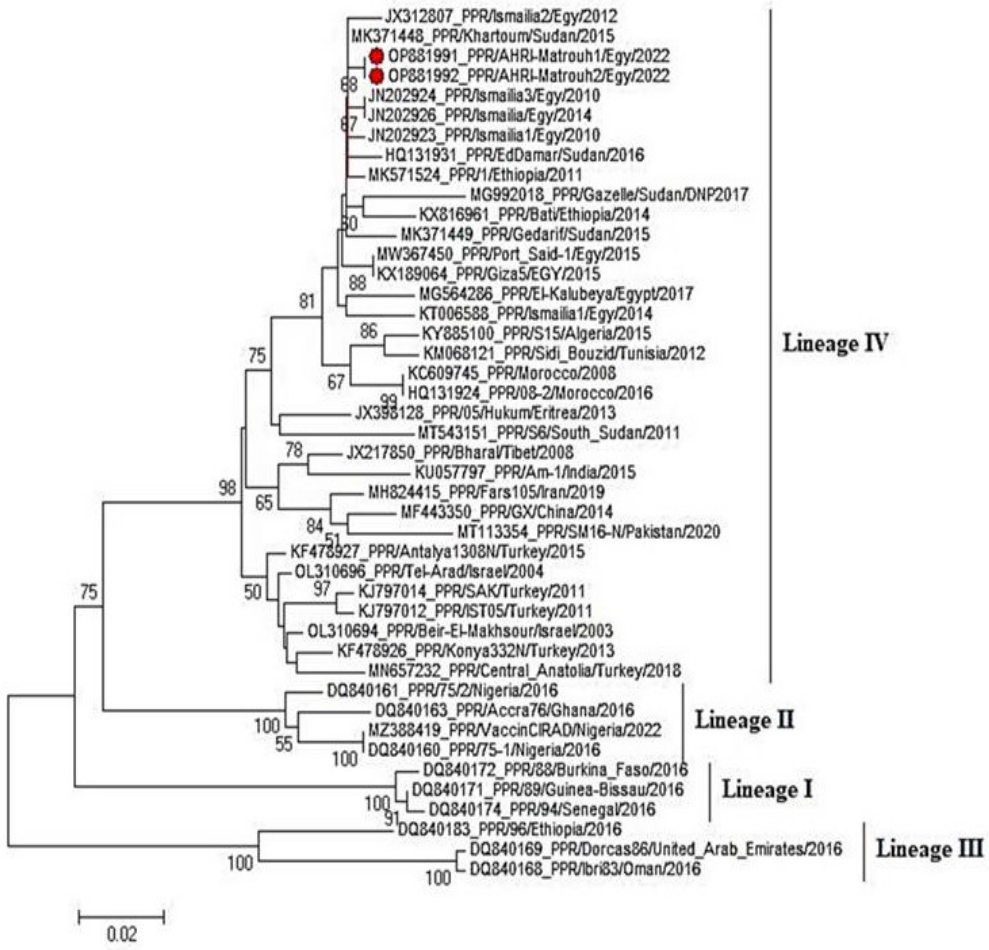

Fig. 1. The phylogenetic tree created using MEGA 7 software with the maximum likelihood method. The confidence level of the neighbor-joining tree was evaluated by bootstrapping using 1000 replicates. The red circles refer to the PPRV strains of our study, with their names and accession numbers.

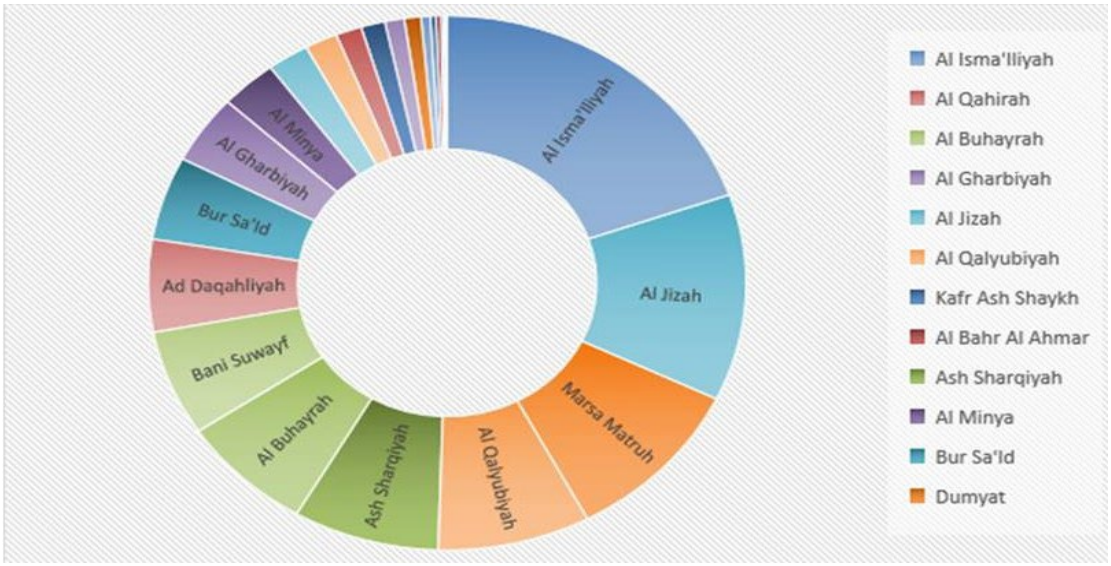

Fig. 2. The prevalence rate of PPRV in the Egyptian governorates from 2012 to 2020.

Ismailia 2012; Giza 2015 and Port Said 2015 with accession numbers JX312807, KX189064 and MW367450, respectively (Figure 1 and Table 3).

DISCUSSION

PPRV is a transboundary notifiable, highly contagious viral animal disease of small ruminants able to raise serious health and economical concerns worldwide (Boyazoglu *et al*., 2005, Cam *et al*., 2005, Cêtre-Sossah *et al*., 2016; Mulumba-Mfumu *et al*., 2021).

We aimed first to assess the prevalence of PPRV in Egypt from 2012 to 2020 retrospectively, then identify the seroprevalences in goat farms in Marsa-Matroh province during an outbreak in 2022, as the most prevelant Egyptian province in PPRV. Moreover, to study the relevant risk factors associated with disease epidemiology, then characterize the circulating viral strain on the molecular level.

Exhaustive data on the PPR outbreaks covering nine years (2012-2020) were selected for retrospective analysis of PPRV situation in Egypt. This data was collected from the WAHIS interface, WOAH, no data was available for the 2021 year.

The study showed the distribution of PPRV outbreaks in sheep and goats in almost Egyptian governorates, mainly in Ismailia, Giza, and Marsa- Matroh, from 2012 to 2020 (Figure 2). The reasons for the spread of PPRV outbreaks in nearly all Egyptian governorates could include transboundary unorganized\unofficial movement of infected and susceptible animals, the presence of suitable climatic conditions (warm and humid) that favor disease epidemiology, a lack or defect in vaccination programs, lack of awareness of PPR in farmers' backyards, and limited funding for disease eradication and control in developing or underdeveloped countries (Parvez *et al*., 2014, Mahamat *et al*., 2018; Ahaduzzaman, 2020).

The analyzed data from 2012 to 2020 on Egyptian PPR outbreaks revealed that the prevalence of PPR disease was the highest from Jul-Dec in 2018 (55.5%), while the prevalence was unrecorded from Jan-Jun 2019 (Figure 3).The prevalence of PPR outbreaks was significantly distributed on the Jul-Dec semester of the year during the period of study; this is due to the PPR disease being more distributed in the warm and humid seasons (Aguilar *et al*., 2018; Ahaduzzaman, 2020; Gao *et al*., 2021; Akwongo *et al*., 2022).

The obtained data classified Marsa-Matroh as one of the governorates with the most significant PPRV outbreaks throughout the research period (Figure 4), which might be owing to its hot and humid environment virtually all year, which is considered a suitable environment for PPRV spread and replication. Furthermore, the variation in the incidence of PPRV outbreaks in Marsa-Matroh throughout the study might be attributed to the random movement of infected animals, the importation of sick animals, and a lack of vaccination during this period (Gao *et al*., 2021; Akwongo *et al*., 2022).

We selected Marsa-Matroh province to conduct the cross-sectional study due to the highest prevalence of PPRV as described previously in our retrospective investigation.

In this study, antibodies were detected against PPRV using competitive ELISA, which revealed that 42.69% of collected sera samples were positive for the presence of the antibodies. These results came in line with the previous studies reported in different countries as Ethiopia (68.18%) and Nigeria (55%) (Dahiru *et al*., 2013; Dubie *et al*., 2022). On the contrary, the current study's seroprevalence is more significant when compared to seroprevalence in other countries such as Turkey (22.4%) and Tanzania (21.1%) (Balamurugan *et al*., 2011; Ozkul *et al*., 2002).

The assessment of risk variables is critical for successful PPRV control and mitigation strategies. Our research focused on age and gender, starting with the age factor, the seroprevalence is higher in goats under the age of six months (47.05%) than in adults (2-3 years) (40.94%). These outcomes agreed with the re-

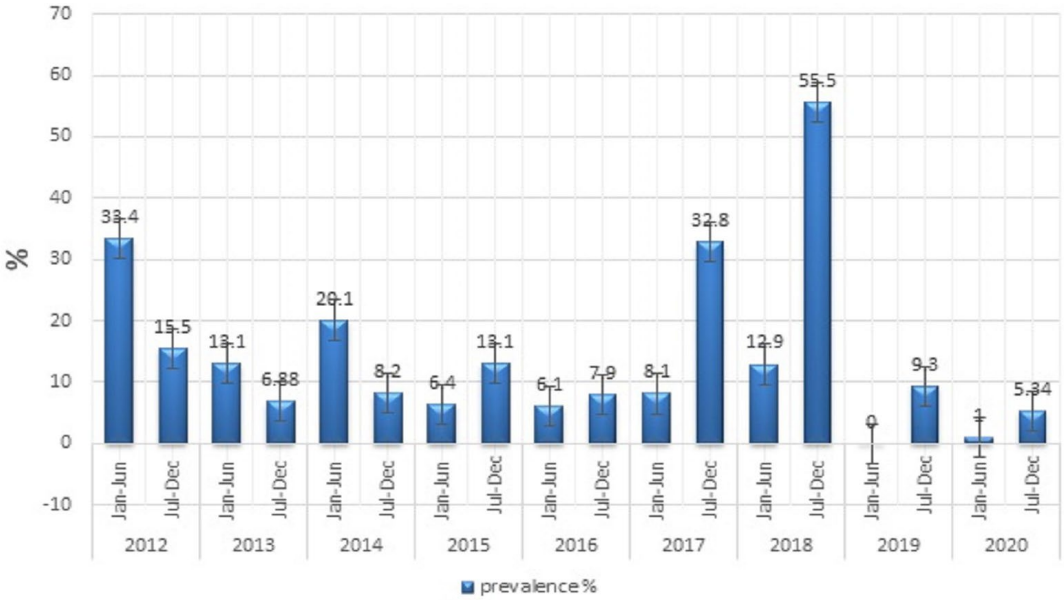

Fig. 3. The PPRV outbreaks from 2012 to 2020 in Egypt concerning the semesters of the year.

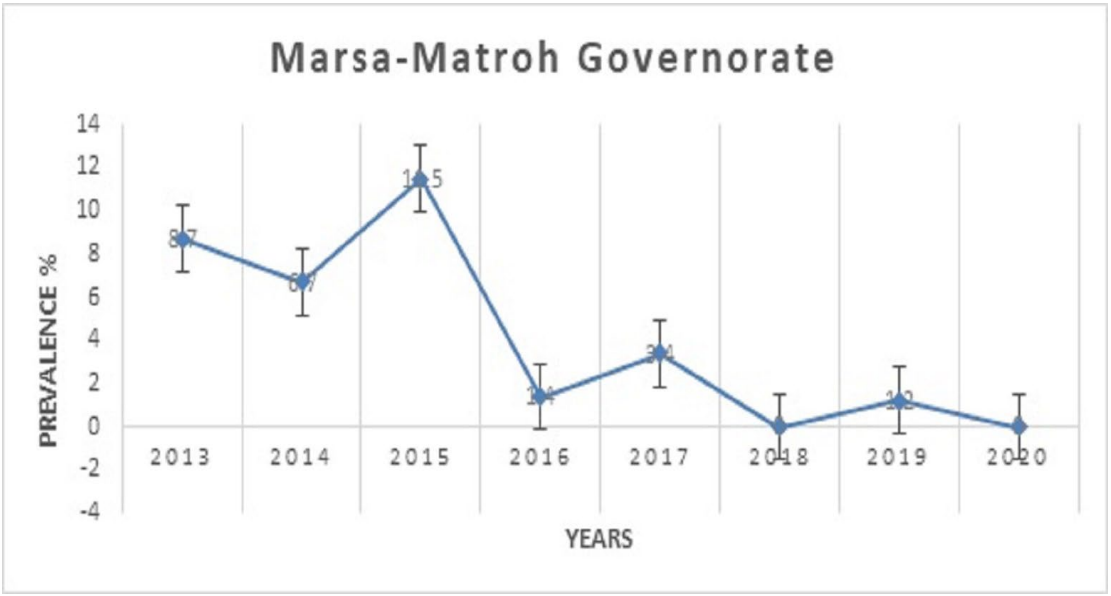

Fig. 4. The PPRV outbreaks in Marsa- Matroh governorate from 2012 to 2020.

Table 3. The percentage of nucleotide sequence identity between the PPRV strains of the current study (PPR/AHRI-Matrouh1/Egy/2022 and PPR/AHRI-Matrouh2/Egy/2022) and reference PPRV strains from the Genbank based on the partial nucleotide sequences of the NP gene

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
1		100.0	97.6	99.2	98.8	99.2	99.2	96.8	88.8	87.1	86.3	88.8	96.0	93.2	98.4	97.2	98.8	87.6	89.6	98.8	97.6	99.2	94.4	98.8	84.3	88.0	80.3	1
2	0.0		97.6	99.2	98.8	99.2	99.2	96.8	88.8	87.1	86.3	88.8	96.0	93.2	98.4	97.2	98.8	87.6	89.6	98.8	97.6	99.2	94.4	98.8	84.3	88.0	80.3	2
3	2.5	2.5		97.6	97.2	97.6	97.6	96.8	87.6	85.9	85.1	87.6	95.2	91.6	96.8	95.6	97.6	86.3	88.4	97.6	96.8	97.6	92.8	97.2	83.1	86.3	80.3	3
4	0.8	0.8	2.5		98.8	99.2	99.2	96.8	88.8	87.1	86.3	88.8	96.0	93.2	98.4	97.2	98.8	87.6	89.6	98.8	97.6	99.2	94.4	98.8	85.1	87.6	80.3	4
5	1.2	1.2	2.9	1.2		98.8	98.8	96.4	88.4	86.7	85.9	88.4	95.6	92.8	98.0	96.8	98.4	87.1	89.2	98.4	97.2	98.8	94.0	98.4	83.9	87.1	79.9	5
6	0.8	0.8	2.5	0.8	1.2		100.0	96.8	88.8	87.1	86.3	88.8	96.0	93.2	98.4	97.2	98.8	87.6	89.6	98.8	97.6	99.2	94.4	98.8	84.3	87.6	81.1	6
7	0.8	0.8	2.5	0.8	1.2	0.0		96.8	88.8	87.1	86.3	88.8	96.0	93.2	98.4	97.2	98.8	87.6	89.6	98.8	97.6	99.2	94.4	98.8	84.3	87.6	81.1	7
8	3.3	3.3	3.3	3.3	3.8	3.3	3.3		87.1	85.5	84.7	87.1	94.4	91.6	96.0	97.2	96.4	85.9	88.0	96.4	95.2	96.8	92.8	96.4	83.5	85.9	79.9	8
9	12.6	12.6	14.1	12.6	13.1	12.6	12.6	14.7		94.0	93.2	96.4	90.0	88.4	88.8	88.4	88.4	94.4	97.2	88.4	88.0	88.8	89.2	88.4	83.9	94.4	81.1	9
10	14.5	14.5	16.0	14.5	15.0	14.5	14.5	16.6	6.3		99.2	92.8	88.4	86.7	87.1	86.7	86.7	99.6	92.8	86.7	86.3	87.1	87.6	86.7	84.3	96.4	81.1	10
11	15.6	15.6	17.1	15.6	16.1	15.6	15.6	17.7	7.3	0.8		92.0	87.6	85.9	86.3	85.9	85.9	98.8	92.0	85.9	85.5	86.3	86.7	85.9	84.3	95.6	80.3	11
12	12.5	12.5	14.0	12.5	13.0	12.5	12.5	14.6	3.7	7.7	8.6		88.8	87.1	88.0	88.4	88.4	93.2	99.2	88.4	88.0	88.8	88.8	88.4	82.7	94.0	79.1	12
13	4.2	4.2	5.0	4.2	4.6	4.2	4.2	5.9	11.1	13.0	14.0	12.6		95.2	96.0	94.8	96.0	88.8	89.6	96.0	95.6	96.0	94.4	95.6	87.1	88.0	81.5	13
14	7.2	7.2	9.1	7.2	7.7	7.2	7.2	9.1	13.0	14.9	16.0	14.5	5.0		93.2	92.8	92.8	87.1	88.0	92.8	92.0	93.6	92.8	92.8	85.5	86.3	79.9	14
15	1.6	1.6	3.3	1.6	2.0	1.6	1.6	4.2	12.6	14.5	15.6	13.6	4.2	7.2		96.4	98.0	87.6	88.8	98.0	96.8	98.4	93.6	98.0	84.3	86.7	80.3	15
16	2.9	2.9	4.6	2.9	3.3	2.9	2.9	2.9	13.1	15.0	16.1	13.0	5.5	7.7	3.8		96.8	87.1	89.2	96.8	96.0	97.2	94.0	96.8	83.1	87.1	80.7	16
17	1.2	1.2	2.5	1.2	1.6	1.2	1.2	3.7	13.0	15.0	16.0	13.0	4.2	7.7	2.0	3.3		87.1	89.2	100.0	97.6	98.8	94.0	98.4	84.7	87.1	79.9	17
18	14.0	14.0	15.6	14.0	14.5	14.0	14.0	16.2	5.9	0.4	1.2	7.3	12.5	14.4	14.0	14.5	14.5		93.2	87.1	86.7	87.6	88.0	87.1	84.7	96.8	81.5	18
19	11.5	11.5	13.0	11.5	12.0	11.5	11.5	13.6	2.9	7.7	8.6	0.8	11.6	13.5	12.5	12.0	12.0	7.3		89.2	88.8	89.6	88.8	89.2	83.5	94.0	79.9	19
20	1.2	1.2	2.5	1.2	1.6	1.2	1.2	3.7	13.0	15.0	16.0	13.0	4.2	7.7	2.0	3.3	0.0	14.5	12.0		97.6	98.8	94.0	98.4	84.7	87.1	79.9	20
21	2.5	2.5	3.3	2.5	2.9	2.5	2.5	5.0	13.6	15.5	16.6	13.5	4.6	8.6	3.3	4.2	2.5	15.0	12.5	2.5		97.6	93.6	97.2	83.9	86.7	79.5	21
22	0.8	0.8	2.5	0.8	1.2	0.8	0.8	3.3	12.5	14.4	15.5	12.5	4.2	6.8	1.6	2.9	1.2	14.0	11.5	1.2	2.5		94.4	98.8	84.3	87.6	80.3	22
23	5.9	5.9	7.7	5.9	6.3	5.9	5.9	7.7	12.0	13.9	15.0	12.5	5.9	7.7	6.8	6.3	6.3	13.5	12.5	6.3	6.8	5.9		94.0	83.9	88.0	79.9	23
24	1.2	1.2	2.9	1.2	1.6	1.2	1.2	3.8	13.1	15.0	16.1	13.0	4.6	7.7	2.0	3.3	1.6	14.5	12.0	1.6	2.9	1.2	6.3		83.9	87.1	80.7	24
25	18.3	18.3	19.9	17.2	18.9	18.3	18.3	19.4	18.8	18.0	18.0	20.4	14.5	16.5	18.3	20.0	17.7	17.5	19.3	17.7	18.8	18.2	18.8	18.9		83.9	80.3	25
26	13.6	13.6	15.6	14.1	14.6	14.1	14.1	16.2	5.9	3.7	4.6	6.4	13.6	15.6	15.1	14.6	14.5	3.3	6.4	14.5	15.1	14.0	13.5	14.6	18.7		80.7	26
27	23.8	23.8	23.7	23.8	24.4	22.6	22.6	24.4	22.5	22.3	23.5	25.5	22.1	24.2	23.8	23.2	24.3	21.8	24.2	24.3	25.0	23.7	24.3	23.2	23.6	23.0		27
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	

OP881991 PPR/AHRI-Matrouh1/Egy/2022
OP881992 PPR/AHRI-Matrouh2/Egy/2022
MG564286 PPR/El-Kalubeya/Egypt/2017
JN202923 PPR/Ismailia1/Egy/2010
JX312807 PPR/Ismailia2/Egy/2012
JN202924 PPR/Ismailia3/Egy/2010
JN202926 PPR/Ismailia/Egy/2014
KY885100 PPR/S15/Algeria/2015
KR781449 PPR/10/Benin/2011
MW492583 PPR/TCN08/Tanzania/2018
KY196465 PPR/AH1608/China/2017
MT072491 PPR/SareKali2/Senegal/2013
OL310696 PPR/Tel-Arad/Israel/2004
KU057797 PPR/Am-1/India/2015
MK371449 PPR/Gedarif/Sudan/2015
KC609745 PPR/Morocco/2008
MW367450 PPR/Port Said-1/Egy/2015
MZ388419 PPR/VaccinCIRAD/Nigeria/2022
MT072468 PPR/Kaala/Guinea/2013
KX189064 PPR/Giza5/EGY/2015
KT006588 PPR/Ismailia1/Egy/2014
MK571524 PPR/1/Ethiopia/2011
MT543151 PPR/S6/South Sudan/2011
HQ131931 PPR/EdDamar/Sudan/2016
DQ840174 PPR/94/Senegal/2016
DQ840163 PPR/Accra76/Ghana/2016
DQ840168 PPR/Ibri83/Oman/2016

sults of several earlier studies, which determined that the disease is more common in young goats than adults due to poor nutrition and poor immunity as predisposing factors for the disease (Sarker and Islam, 2011; Rahman *et al*., 2011; Islam *et al*., 2012; Ahaduzzaman, 2020) However, our results contradicted with some former studies, in which the authors indicated that the prevalence increased with age and claimed this to the maternal antibodies (Abubakar *et al*., 2009; Saeed *et al*., 2018).

Referring to Sex, females have a more significant disease incidence than males. According to our data, seropositivity is 47.86 % in female goats and 35.17% in male goats. These results support the findings of Shuaib (2011); Abdalla *et al*. (2012); Saeed *et al*. (2018); Ahaduzzaman, (2020) and Akwongo *et al*. (2022). The higher prevalence in females than males may be attributed to the physiological differences resulting from production and reproduction stress, which makes females more vulnerable to infection. In contrast, most males are castrated for meat production (Dubie *et al*., 2022). Besides, females are maintained for extended periods for reproduction, increasing the likelihood of infection (Acharya *et al*., 2018). On the other hand, our findings did not correlate with a prior study that demonstrated that male goats have higher seroprevalence than females due to the genetic variation of the animals (Sarker and Islam, 2011; Parvez, 2014).

We also detected the PPRV antigen using ELISA in the collected nasal swab samples, which is considered the premier choice for PPRV antigen detection (Halecker *et al*., 2020). The current study analysis indicated 92.2% positive cases. These results did not match the results of the previous research, in which the authors reported a low antigen detection percentage reached 27.53% and 44.2% (Saritha *et al*., 2015; Ahmed *et al*., 2021).

According to our findings, goats of young age, less than six months, have a greater incidence rate of antigen detection (97.72%) than adults (90%), and females have a higher positive rate (95.60%) than males (87.30%). Overall, the antigen detection results corroborate the seroprevalence findings.

By molecular diagnosis, all tested samples were positive for PPRV, the same assay was used previously in different studies, in which the authors concluded that using NP gene-based PCR is more sensitive in the molecular diagnosis of PPRV (Kumar *et al*., 2014).

PPRVs are divided into four genetically distinct lineages (I, II, III, and IV) (Kerur *et al*., 2008), The PPRV NP gene sequence analysis is best suited for the phylogenetic differentiation of these viruses and gives a comprehensive perspective of PPRV molecular epidemiology (Munir *et al*., 2012). The phylogenetic analysis in the current study identified our strains as PPRV linage IV, which grouped with the previous Egyptian strains in one cluster that included PPRV reported in Ethiopia (2011) and Sudan (2016). These findings followed prior research in which the authors claimed that the circulating Egyptian strains belonged to the Ethiopian strain of lineage IV (El Ashmawy *et al*., 2018). The transportation of live animals from Ethiopia and Sudan to Egypt is thought to have contributed to the spread of this virus throughout North and East Africa. This is corroborated by the strong relationship between the PPRV IV lineage in Egypt and North Africa and the PPRV lineage first discovered in Sudan and Ethiopia (Hekal *et al*., 2019; Ahmed *et al*., 2021).

CONCLUSION

According to the findings of this study, the current circulating strain in most recent outbreak is PPRV lineage IV which is genetically closely related to Ethiopia and Sudan strains. These findings highlight the significance of animal movement regulations. PPRV monitoring programs are required to continous monitoring the circulating strains in Egypt, and a vaccination plan should be developed in Egyptian endemic regions.

ACKNOWLEDGMENTS

The authors would like to extend their appreciation and thanks to Animal Health Research Institute, Agriculture Research Center, Egypt, for assistance and for providing all laboratory facilities till the completion of this study.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abdalla, A.S., Majok, A.A., Elmalik, K.H., Ali, A.S., 2012. Seroprevalence of Peste des Petits ruminants virus (PPRV) in small ruminants in Blue Nile, Gadaref and North Kordofan States of Sudan. J. Public Health Epidem. 4, 59-64.

Abubakar, M., Jamal, S.M., Arshed, M.J., Hussain, M., Ali, Q., 2009. Peste des petits ruminants virus (PPRV) infection; its association with species, seasonal variations, and geography. Trop. Anim. Health Prod. 41, 1197–1202.

Abubakar, M., Ashiq, S., Hussain, Z., Hussain, M., Saleha, S., Arshad, M. Zahoor, A., 2011. Comparison of antigen detection methods of peste des petits ruminants' virus in clinical samples of small ru-

minants. Bulgarian Journal of Veterinary Medicine 14, 103–108.
Acharya, N., Poudel, S.P., Acharya, K.P., 2018. Cross-sectional seroprevalence study of Peste des Petits Ruminants (PPR) in goats of Syangja and Kaski districts of Nepal. Virus Dis. 29, 173-179.
Aguilar, X.F., Fine, A.E., Pruvot, M., Njeumi, F., Walzer, C., Kock, R., Shiilegdamba, E., 2018. PPR virus threatens wildlife conservation. Science 362,165-166.
Ahaduzzaman, Md., 2020. Peste des petits ruminants (PPR) in Africa and Asia: A systematic review and meta-analysis of the prevalence in sheep and goats between 1969 and 2018. Vet. Med. Sci. 2020, 1–21.
Ahmed, S., Hosny, W.A.E., Mahmoud, M., Mahmoud, M.A.E., 2021. Isolation and identification of peste des petits ruminants virus from goats in Egyptian governorates. Vet. World 14, 926-932.
Akwongo, C.J., Melvyn, Q., Charles, B., 2022. Prevalence, risk factors for exposure, Socio-Economic impact of Peste Des Petits ruminants in Karenga district, Karamoja region, Uganda. Pathogen 54, 1–18.
Alemu, B., Gari, G., Libeau, G., Kwiatek, O., Kidane, M., Belayneh, R., Siraw, B., Wieland, B., Asfaw, W., Abdi, RD., 2019. Molecular detection and phylogenetic analysis of Peste des petits ruminants virus circulating in small ruminants in eastern Amhara region, Ethiopia. BMC Vet. Res. 15, 84.
Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipmanl, D.J., 1990. Basic Local Alignment Search Tool. J. Mol. Biol. 215, 403-410.
Balamurugan, P., Saravanan, P., Bhanuprakash, V., Krishnamoorthy, P., Singh, R., 2011. Seroepidemiological study of peste des petits ruminants in sheep and goats in India between 2003 and 2009. Rev. Sci. Tech. Off. Int. Epiz. 30, 889–96.
Boyazoglu, J., Hatziminaoglou, I., Morand-Fehr, P., 2005. The role of the goat in society: past, present, and perspectives for the future. Small Rumin. Res. 60, 13-23.
Cam, Y., Gencay, A., Beyaz, L., Atalay, O., Atasever, A., Ozkul, A., Kibar, M., 2005. Peste des petits ruminants in a sheep and goat flock in Kayseri province, Turkey Vet. Rec. 157, 523.
Cêtre-Sossah, C., Kwiatek, O., Faharoudine, A., Soulé, M., Moutroifi, Y., Vrel, M.A. 2016. Impact and epidemiological investigations into the incursion and spread of Peste des petits ruminants in the Comoros Archipelago: An increased threat to surrounding Islands. Transbound. Emerg. Dis. 63, 452–459.
Chard, L.S., Bailey, D.S., Dash, P., Banyard, A.C., Barrett, T., 2008. Full genome sequences of two virulent strains of peste-des-petits ruminants virus, the Côte d'Ivoire 1989 and Nigeria 1976 strains. Virus Res. 1361, 192-197.
Dahiru, A., Baba, S., Egwu, O., 2013. Seroprevalence of peste des petits ruminants among domestic small and large ruminants in the semi-arid region of northeastern Nigeria. Vet. World. 10, 807-11.
Dilli, H., Geidam, Y., Egwu, G., 2011. Peste de petits ruminants in Nigeria: A review. Niger. Vet. J., 32, 2.
Dubie, T., Dagnew, B., Gelo, E., Negash, W., Hussein, F., Woldehana, M., 2022. Seroprevalence and associated risk factors of peste des petits ruminants among ovine and caprine in selected Afar region, Ethiopia districts. BMC Vet. Res. 18, 429.
El Ashmawy, W.R., Bazid, A.I., Aboelkhair, M., Sakr, M.A., Fayed, A.M., Fawzy, M., 2018. Molecular epidemiological study on peste des petits ruminants in Egypt 2015. Res. 55, 4, 205–212.
Elliot, J.L., Donald, M.D., Robert, C.H., Richard, J.O., Stuart, G.S., Donald, B.S., 2018.Virus taxonomy: the database of the International Committee on Taxonomy of Viruses (ICTV). Nucleic Acids Res. 46, D708-D717.
FAO, 2015. Global Strategy for the Control and Eradication of PPR. http://www.fao.org/3/ai4460e.pdf.
Fournié, G., Waret-Szkuta, A., Camacho, A., Yigezu, L.M., Pfeiffer, D.U., Roger, F., 2018. A dynamic model of transmission and elimination of Peste des petits ruminants in Ethiopia. Proceed. Nat. Acad. Sci. 115, 8454–8459.
Gao, S., GuoYong, X., Zan, Z., JiaNing, L., LiYa, H., HaoNing, W., XiaoLong, W., 2021. Transboundary spread of peste des petits ruminants virus in western China: A prediction model. PLoS One 16, e0257898.
Halecker, S., Joseph, S., Mohammed, R., Wernery, U., Mettenleiter, T.C., Beer, M., Hoffmann, B., 2020. Comparative evaluation of different antigen detection methods for detecting peste des petits ruminants virus. Transbound. Emerg. Dis. 67, 2881-2891.
Hekal, S.H.A., Al-Gaabary, M.H., El-Sayed, M.M., Sobhy, H.M., Fayed, A.A.A., 2019. Seroprevalence of some infectious transboundary diseases in cattle imported from Sudan to Egypt. J. Adv. Vet. Anim. Res. 6, 92-99.
Islam, M.S., Khan, M.S.I., Kader, H.A., Begum, M.R., Asgar, M.A., 2012. Prevalence of PPR of goats and their response to antibiotic treatment at Mirzaganj Upazila of Patuakhali District. J. Environ. Sci. Natural Resour. 5, 81–184.
Kerur, N., Jhala, M.K., Joshi, C.G., 2008. Genetic characterization of Indian peste des petits ruminants virus (PPRV) by sequencing and phylogenetic analysis of fusion protein and nucleoprotein gene segments. Res. Vet. Sci. 85, 176–83.
Kgotlele, T., Macha, E.S., Kasanga, C.J., Kusiluka, L.J.M., Karimuribo, E.D., Van Doorsselaere, J., Wensman, J.J., Munir, M., and Misinzo, G., 2014. Partial Genetic Characterization of Peste Des Petits Ruminants Virus from Goats in Northern and Eastern Tanzania. Transboundary and Emerging Diseases Published by Blackwell Verlag GmbH. 61, 56–62.
Kumar, K.S., Babu, A., Sundarapandian, G., Roy, P., Thangavelu, A., Kumar, K.S., Arumugam, R., Chandran, N.D., Muniraju, M., Mahapatra, M., Banyard, A.C., Manohar, B.M., Parida, S., 2014. Molecular characterization of lineage IV peste des petits ruminants virus using multi-gene sequence data. Vet. Microbiol. 174, 39-49.
Libeau, G., Préhaud, C., Lancelot, R., Colas, F., Guerre, L., Bishop, D.H., Diallo, A., 1995. Developing a competitive ELISA for detecting antibodies to the Peste des Petits Ruminants virus using a recombinant nucleoprotein. Res. Vet. Sci. 58, 50-5.
Mahamat, O., Doungous, T., Kebkiba, B., Oumar, H.A., Oussiguéré, A., Yacoub, A.H., 2018. Seroprevalence, geographical distribution, and risk factors of Peste des petits ruminants in the Republic of Chad. J. Adv. Vet. Anim. Res. 5, 420-425.
Mahapatra, M., Pachauri, R., Subramaniam, S., Banyard, A.C., ChandraSekar, S., Ramakrishnan, M.A., Njeumi, F., Muthuchelvan, D., Parida, S., 2021. Ongoing Assessment of the Molecular Evolution of Peste Des Petits Ruminants Virus Continues to Question Viral Origins. Viruses 13, 2144.
Mulumba-Mfumu, L.K., Mana, M., Adama, D., Brian, C., Augustin, T., Jean, P.M., Felix, N., Satya, P., 2021. Retrospective Characterization of Initial Peste des petits ruminants Outbreaks (2008–2012) in the Democratic Republic of the Congo. Viruses 13, 2373.2-14.
Munir, M., Zohari, S., Suluku, R., LeBlanc, N., Kanu, S., Sankoh, F.A.R., Ståhl, K., 2012. Genetic characterization of peste des petits ruminants virus, Sierra Leone. Emerg. Infect. Dis. 18, 193-195.
Nafea, M.R., Elbakry, M., Shahein, M., Farag, G.K., Abdallah, F., Ali, A.A.H., 2019. Virological and molecular studies on peste des petits ruminants virus (PPRV) in small ruminants and camels in Egypt between 2017 and 2018. Adv. Anim. Vet. Sci. 7, 12-18.
Ozkul, A., Akca, Y., Alkan, F., Barrett, T., Karaoglu, T., Dagalp, SB., Anderson, J., Yesil- bag K, Cokcaliskan, C., Gencay, A., Burgu, I., 2002. Prevalence, distribution, and host range of Peste des petits ruminants' virus, Turkey. Emerg. Infec. Dis. 8, 708-712.
Parida, S., Muniraju, M., Altan, E., Baazizi, R., Raj, G.D., Mahapatra, M., 2016. The emergence of PPR and its threat to Europe. Small Rum. Res. 142, 16–21.
Parvez, M.A., Khatun, R., Al Noman, M.A., 2014. Prevalence and associated risk factors of Peste des petits ruminants (PPR) in goats in Chittagong district, Bangladesh. Res. J. Vet. Pract. 2, 14–17.
Rahman, M.A., Shadmin, I., Noor, M., Parvin, R., Chowdhury, E.H., Islam, M.R., 2011. Peste des petits ruminants virus infection of goats in Bangladesh Pathological investigation, molecular detection and isolation of the virus. Bangladesh Vet. 28, 1–7.
Saeed, F., Abdel-Aziz, S., Gumaa, M., 2018. Seroprevalence and Associated Risk Factors of Peste des Petits Ruminants among Sheep and Goats in Kassala State, Sudan. Open J. Anim.Sci. 8, 381-395.
Saritha, G., Shobhamani, B., Rajak, K.K., Sreedevi, B., 2015. Detection and confirmation of PPR virus antigen in sheep and goats by sandwich-ELISA and RT-PCR in Andhra Pradesh, India. J. Adv. Vet. Anim. Res. 2, 210–212.
Sarker, S., Islam, H., 2011. Prevalence and risk factors assessment of PPR in goats in Rajshahi, Bangladesh. Vet. World 4, 546-549.
Shuaib, Y.A., 2011. Peste des petits ruminants in Sheep in Sudan: A study on seroprevalence and risk factors, MSc thesis, Graduate School of Addis Ababa University, Department of Public health, Faculty of Veterinary Medicine, Freie Universitat Berlin and Sudan University of Science and Technology, Sudan.
Tamura, K., Stecher, G., Peterson, D., Filipski, A. Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position-specific gap penalties, and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
Wagdy, R., ElAshmawy, A.I., Bazid, M.A., Mostafa, A., Sakr, A.M., Fayed, M.F., 2018. Molecular epidemiological study on Peste des Petits Ruminants. In: Egypt 2015. Slov. Vet. Res. 55, 205-212.